

WHAT IS CLAIMED IS:

- 1                    1.        An isolated protein comprising a HER-2/neu extracellular domain  
2 fused to a HER-2/neu phosphorylation domain, wherein the protein is capable of  
3 producing an immune response in a warm-blooded animal.
- 1                    2.        The protein of claim 1, wherein the protein has a sequence at least  
2 80% identical to the sequence of SEQ ID NO:6, or wherein the protein comprises a  
3 sequence at least 80% identical to the sequence of SEQ ID NO:3 fused to a sequence at  
4 least 80% identical to the sequence of SEQ ID NO:4.
- 1                    3.        The protein of claim 1, wherein the protein comprises a sequence at  
2 least 80 % identical to the sequence of SEQ ID NO:3 directly fused to an amino acid  
3 sequence at least 80% identical to the sequence inclusive of Gln 991 to Val 1256 of SEQ  
4 ID NO:2, or wherein the protein comprises a sequence at least 80 % identical to the  
5 sequence of SEQ ID NO:3 fused to the amino acid sequence at least 80% identical to the  
6 sequence inclusive of Gln 991 to Val 1256 of SEQ ID NO:2.
- 1                    4.        The protein of claim 1, wherein the protein comprises a sequence at  
2 least 80% identical to the sequence of SEQ ID NO:8 directly fused to a sequence at least  
3 80% identical to the sequence of SEQ ID NO:4, or wherein the protein comprises a  
4 sequence at least 80% identical to the sequence of SEQ ID NO:8 fused to a sequence at  
5 least 80% identical to the sequence of SEQ ID NO:4.
- 1                    5.        The protein of claim 1, wherein the protein comprises a sequence at  
2 least 80% identical to the sequence of SEQ ID NO:8 directly fused to the amino acid  
3 sequence inclusive of Gln 991 to Val 1256 of SEQ ID NO:2, or wherein the protein  
4 comprises a sequence at least 80% identical to the sequence of SEQ ID NO:8 fused to a  
5 sequence at least 80% identical to the amino acid sequence inclusive of Gln 991 to Val  
6 1256 of SEQ ID NO:2.
- 1                    6.        The protein of claim 1, wherein the HER-2/neu extracellular  
2 domain is fused to the HER-2/neu phosphorylation domain via a chemical linker.
- 1                    7.        The protein of claim 6, wherein the chemical linker is an amino  
2 acid linker.

1                    8.        A nucleic acid molecule encoding the protein of claim 1.

1                    9.        A viral vector comprising a polynucleotide sequence encoding the  
2        protein of claim 1.

1                    10.     A pharmaceutical composition comprising the protein of claim 1,  
2     and a pharmaceutically acceptable carrier or diluent.

1                    11.    The pharmaceutical composition of claim 10, wherein the  
2    pharmaceutical composition is a vaccine.

1                    12.    The pharmaceutical composition of claim 10, further comprising an  
2 immunostimulatory substance.

1 13. The pharmaceutical composition of claim 12, wherein the protein is  
2 presented in an oil-in-water emulsion.

1                    14.     The pharmaceutical composition of claim 12, wherein the  
2     immunostimulatory substance is SBAS2, 3D-MPL, QS21, or a combination of 3D-MPL  
3     and QS21.

1            15.    A pharmaceutical composition comprising the nucleic acid  
2 molecule of claim 8, and a pharmaceutically acceptable carrier or diluent.

1                    16.    The pharmaceutical composition of claim 15, wherein the  
2    pharmaceutical composition is a vaccine.

1                 17. The pharmaceutical composition of claim 15, further comprising an  
2 immunostimulatory substance.

1                18. The pharmaceutical composition of claim 15, wherein the nucleic  
2 acid molecule is a DNA molecule.

1 19. A method for eliciting or enhancing an immune response to HER-  
2 2/neu protein, the method comprising the step of administering to a warm-blooded animal  
3 the protein of claim 1 in an amount effective to elicit or enhance the immune response.



4 ID NO:2, or wherein the protein comprises a sequence at least 80% identical to the  
5 sequence of SEQ ID NO:3 fused to a sequence at least 80% identical to the amino acid  
6 sequence inclusive of Gln 991 to Arg 1049 of SEQ ID NO:2.

1                    29.     The protein of claim 26, wherein the protein comprises a sequence  
2     at least 80% identical to the sequence of SEQ ID NO:8 directly fused to a sequence at  
3     least 80% identical to the sequence of SEQ ID NO:5, or wherein the protein comprises a  
4     sequence at least 80% identical to the sequence of SEQ ID NO:8 fused to a sequence at  
5     least 80% identical to the sequence of SEQ ID NO:5.

1                                30.     The protein of claim 26, wherein the protein comprises a sequence  
2     at least 80% identical to the sequence of SEQ ID NO:8 directly fused to a sequence at  
3     least 80% identical to the amino acid sequence inclusive of Gln 991 to Arg 1049 of SEQ  
4     ID NO:2, or wherein the protein comprises a sequence at least 80% identical to the  
5     sequence of SEQ ID NO:8 fused to a sequence at least 80% identical to the amino acid  
6     sequence inclusive of Gln 991 to Arg 1049 of SEQ ID NO:2.

1 31. The protein of claim 26, wherein the HER-2/neu extracellular  
2 domain is fused to the fragment of the HER-2/neu phosphorylation domain via a chemical  
3 linker.

1 32. The protein of claim 31, wherein the chemical linker is an amino  
2 acid linker.

1 33. A nucleic acid molecule encoding the protein of claim 26.

1 34. A viral vector comprising a polynucleotide sequence encoding the  
2 protein of claim 26.

1                    35.    A pharmaceutical composition comprising the protein of claim 26,  
2    and a pharmaceutically acceptable carrier or diluent.

1                    36. The pharmaceutical composition of claim 35, wherein the  
2 pharmaceutical composition is a vaccine.

1                    37.    The pharmaceutical composition of claim 35, further comprising an  
2 immunostimulatory substance.

1                    38.     The pharmaceutical composition of claim 37, wherein the protein is  
2     presented in an oil-in-water emulsion.

1                    39.     The pharmaceutical composition of claim 37, wherein the  
2 immunostimulatory substance is SBAS2, 3D-MPL, QS21, or a combination of 3D-MPL  
3 and QS21.

1                    40.    A pharmaceutical composition comprising the nucleic acid  
2    molecule of claim 33, and a pharmaceutically acceptable carrier or diluent.

1                    41.    The pharmaceutical composition of claim 40, wherein the  
2    pharmaceutical composition is a vaccine.

1                    42.    The pharmaceutical composition of claim 40, further comprising an  
2 immunostimulatory substance.

43. The pharmaceutical composition of claim 40, wherein the nucleic acid molecule is a DNA molecule.

1 44. A method for eliciting or enhancing an immune response to HER-  
2 2/neu protein, the method comprising the step of administering to a warm-blooded animal  
3 the protein of claim 26 in an amount effective to elicit or enhance the immune response.

1                    45.    The method of claim 44, wherein the protein is administered in the  
2    form of a vaccine.

1                    46.     A method for eliciting or enhancing an immune response to HER-  
2     2/neu protein, the method comprising the step of administering to a warm-blooded animal  
3     the nucleic acid molecule of claim 33 in an amount effective to elicit or enhance the  
4     immune response.

1                    47.    The method of claim 46, wherein the nucleic acid molecule is in  
2    the form of a vaccine.

1                    48.     The method of claim 46, wherein the step of administering  
2     comprises transfecting cells of the warm-blooded animal *ex vivo* with the nucleic acid  
3     molecule and subsequently delivering the transfected cells to the warm-blooded animal.

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1 49. A method for eliciting or enhancing an immune response to HER-  
2 2/neu protein, the method comprising the step of administering to a warm-blooded animal  
3 the viral vector of claim 34 in an amount effective to elicit or enhance the immune  
4 response.

1 50. The method of claim 49, wherein the step of administering  
2 comprises infecting cells of the warm-blooded animal *ex vivo* with the viral vector and  
3 subsequently delivering the infected cells to the warm-blooded animal.

1 51. An isolated protein comprising a HER-2/neu extracellular domain  
2 fused to a HER-2/neu intracellular domain, wherein the protein is capable of producing an  
3 immune response in a warm-blooded animal.

1 52. The protein of claim 51, wherein the protein comprises a sequence  
2 at least 80% identical to the sequence of SEQ ID NO:3 fused directly to a sequence at  
3 least 80% identical to the amino acid sequence inclusive of Lys 676 to Val 1255 in SEQ  
4 ID NO:1, or wherein the protein comprises a sequence at least 80% identical to the  
5 sequence of SEQ ID NO:3 fused to a sequence at least 80% identical to the amino acid  
6 sequence inclusive of Lys 676 to Val 1255 of SEQ ID NO:1 via at least one of a chemical  
7 or amino acid linking group.

1 53. The protein of claim 51, wherein the protein comprises a sequence  
2 at least 80% identical to the sequence of SEQ ID NO:3 directly fused to a sequence at  
3 least 80% identical to the amino acid sequence inclusive of Lys 677 to Val 1256 of SEQ  
4 ID NO:2, or wherein the protein comprises a sequence at least 80% identical to the  
5 sequence of SEQ ID NO:3 fused to a sequence at least 80% identical to the amino acid  
6 sequence inclusive of Lys 677 to Val 1256 of SEQ ID NO:2 via at least one of a chemical  
7 or amino acid linking group.

1 54. The protein of claim 51, wherein the protein comprises a sequence  
2 at least 80% identical to the sequence of SEQ ID NO:8 directly fused to a sequence at  
3 least 80% identical to the amino acid sequence inclusive of Lys 676 to Val 1255 of SEQ  
4 ID NO:1, or wherein the protein comprises a sequence at least 80% identical to the  
5 sequence of SEQ ID NO:8 fused to a sequence at least 80% identical to the amino acid

6 sequence inclusive of Lys 676 to Val 1255 of SEQ ID NO:1 via at least one of a chemical  
7 or amino acid linking group.

1                    55.        The protein of claim 51, wherein the protein comprises a sequence  
2        at least 80% identical to the sequence of SEQ ID NO:8 directly fused to a sequence at  
3        least 80% identical to the amino acid sequence inclusive of Lys 677 to Val 1256 of SEQ  
4        ID NO:2, or wherein the protein comprises a sequence at least 80% identical to the  
5        sequence of SEQ ID NO:8 fused to a sequence at least 80% identical to the amino acid  
6        sequence inclusive of Lys 677 to Val 1256 of SEQ ID NO:2 via at least one of a chemical  
7        or amino acid linking group.

1                    56.    The protein of claim 51, wherein the HER-2/neu extracellular  
2    domain is fused to the HER-2/neu intracellular domain via a chemical linker.

1  
2 acid linker.

57. The protein of claim 56, wherein the chemical linker is an amino

1 58. A nucleic acid molecule encoding the protein of claim 51.

1 59. A viral vector comprising a polynucleotide sequence encoding the  
2 protein of claim 51.

1                    60.    A pharmaceutical composition comprising the protein of claim 51,  
2    and a pharmaceutically acceptable carrier or diluent.

1                    61.    The pharmaceutical composition of claim 60, wherein the  
2    pharmaceutical composition is a vaccine.

1                    62.    The pharmaceutical composition of claim 60, further comprising an  
2 immunostimulatory substance.

1                    63.    The pharmaceutical composition of claim 62, wherein the protein is  
2    presented in an oil-in-water emulsion.

1                    64.     The pharmaceutical composition of claim 62, wherein the  
2 immunostimulatory substance is SBAS2, 3D-MPL, QS21, or a combination of 3D-MPL  
3 and QS21.

1                    65.    A pharmaceutical composition comprising the nucleic acid  
2 molecule of claim 58, and a pharmaceutically acceptable carrier or diluent.

1                    66.    The pharmaceutical composition of claim 65, wherein the  
2 pharmaceutical composition is a vaccine.

1                    67.    The pharmaceutical composition of claim 65, further comprising an  
2 immunostimulatory substance.

1                    68.    The pharmaceutical composition of claim 65, wherein the nucleic  
2 acid molecule is a DNA molecule.

1                    69.    A method for eliciting or enhancing an immune response to HER-  
2 2/neu protein, the method comprising the step of administering to a warm-blooded animal  
3 the protein of claim 51 in an amount effective to elicit or enhance the immune response.

1                    70.    The method of claim 69, wherein the protein is administered in the  
2 form of a vaccine.

1                    71.    A method for eliciting or enhancing an immune response to HER-  
2 2/neu protein, the method comprising the step of administering to a warm-blooded animal  
3 the nucleic acid molecule of claim 58 in an amount effective to elicit or enhance the  
4 immune response.

1                    72.    The method of claim 71, wherein the nucleic acid molecule is in  
2 the form of a vaccine.

1                    73.    The method of claim 71, wherein the step of administering  
2 comprises transfecting cells of the warm-blooded animal *ex vivo* with the nucleic acid  
3 molecule and subsequently delivering the transfected cells to the warm-blooded animal.

1                    74.    A method for eliciting or enhancing an immune response to HER-  
2 2/neu protein, the method comprising the step of administering to a warm-blooded animal  
3 the viral vector of claim 59 in an amount effective to elicit or enhance the immune  
4 response.



75. The method of claim 74, wherein the step of administering comprises infecting cells of the warm-blooded animal *ex vivo* with the viral vector and subsequently delivering the infected cells to the warm-blooded animal.

76. A method for inhibiting the development of a cancer in a patient, the method comprising the step of administering to a patient an effective amount of a fusion polypeptide according to claim 1, 26, or 51 and thereby inhibiting the development of a cancer in the patient.

77. A method for inhibiting the development of a cancer in a patient, the method comprising the step of administering to a patient an effective amount of a polynucleotide according to claim 8, 33, or 58 and thereby inhibiting the development of a cancer in the patient.

78. A method for inhibiting the development of a cancer in a patient, the method comprising the step of administering to a patient an effective amount of an antigen-presenting cell that expresses a fusion polypeptide according to claim 1, 26, or 51, and thereby inhibiting the development of a cancer in the patient.

79. A method according to claim 78, wherein the antigen-presenting cell is a dendritic cell.

80. A method according to any one of claims 76-79, wherein the cancer is breast, ovarian, colon, lung or prostate cancer.

81. A method for removing tumor cells from a biological sample, the method comprising the step of contacting a biological sample with T cells that specifically react with a HER-2/neu fusion protein, wherein the fusion protein comprises an amino acid sequence that is encoded by a polynucleotide sequence selected from the group consisting of:

(i) polynucleotides recited in any one of SEQ ID NO:8, 33, or 58; and

(ii) complements of the foregoing polynucleotides;  
wherein the step of contacting is performed under conditions and for a time sufficient to permit the removal of cells expressing the antigen from the sample.

1 82. A method according to claim 81, wherein the biological sample is  
2 blood or a fraction thereof.

1 83. A method for inhibiting the development of a cancer in a patient,  
2 comprising the step of administering to a patient a biological sample treated according to  
3 the method of claim 81.

1 84. A method for stimulating and/or expanding T cells specific for a  
2 HER-2/neu fusion protein, the method comprising the step of contacting T cells with one  
3 or more of:

- 4 (i) a fusion protein according to claims 1, 26, or 51;  
5 (ii) a polynucleotide encoding such a fusion protein; or  
6 (iii) an antigen presenting cell that expresses such a fusion protein;  
7 under conditions and for a time sufficient to permit the stimulation and/or  
8 expansion of T cells.

1 85. An isolated T cell population, comprising T cells prepared  
2 according to the method of claim 84.

1 86. A method for inhibiting the development of a cancer in a patient,  
2 the method comprising the step of administering to a patient an effective amount of a T  
3 cell population according to claim 85.

1 87. A method for inhibiting the development of a cancer in a patient,  
2 the method comprising the steps of:

3 (a) incubating CD4<sup>+</sup> and/or CD8<sup>+</sup> T cells isolated from a patient with  
4 at least one component selected from the group consisting of:

- 5 (i) a fusion protein according to claims 1, 26, or 51;  
6 (ii) a polynucleotide encoding such a fusion protein; and  
7 (iii) an antigen-presenting cell that expresses such a fusion  
8 protein;

9 such that T cells proliferate; and

10 (b) administering to the patient an effective amount of the proliferated  
11 T cells, thereby inhibiting the development of a cancer in the patient.

1 88. A method for inhibiting the development of a cancer in a patient,  
2 the method comprising the steps of:

3 (a) incubating CD4<sup>+</sup> and/or CD8<sup>+</sup> T cells isolated from a patient with  
4 at least one component selected from the group consisting of:

- 5 (i) a fusion protein according to claims 1, 26, or 51;  
6 (ii) a polynucleotide encoding such a fusion protein; and  
7 (iii) an antigen-presenting cell that expresses such a fusion  
8 protein;

9 such that T cells proliferate;

10 (b) cloning at least one proliferated cell; and

11 (c) administering to the patient an effective amount of the cloned T  
12 cells, thereby inhibiting the development of a cancer in the patient.

1 89. A method of making a fusion protein according to claims 1, 26, or  
2 51, the method comprising the steps of:

3 (a) introducing into a cell an expression vector comprising a  
4 polynucleotide according to claims 8, 33, or 58;

5 (b) culturing the transfected cell; and

6 (c) purifying the expressed protein.

1 90. The method of claim 89, wherein the cell is a CHO cell.

1 91. The method of claim 89, wherein the cell is cultured in suspension,  
2 under serum-free conditions.

1 92. The method of claim 89, wherein the expressed protein is purified  
2 by a two-step procedure, the procedure comprising:

3 (a) anion exchange chromatography on Q sepharose High Performance  
4 Columns; and

5 (b) hydrophobic chromatography on Phenyl Sepharose 6 Fast Flow  
6 low substitution.

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